

Claims:

1. A method for detecting the presence of a target nucleic acid in a sample comprising:
treating a sample containing nucleic acid with an agent that modifies
unmethylated cytosine;
5 providing to the treated sample a detector ligand in the form of an intercalating
nucleic acid (INA) capable of binding to a target region of nucleic acid, and allowing
sufficient time for the detector ligand to bind to the target nucleic acid; and
detecting binding of the detector ligand to nucleic acid molecule in the sample to
indicate the presence of the target nucleic acid.
- 10 2. The method according to claim 1 wherein the nucleic acid is obtained from a genome
of an eukaryote, a prokaryote, virus, mitochondrial nucleic acid, nucleic acid found in
other cellular organelles, extracellular nucleic acid, DNA and RNA forms and natural
or artificial derivatives of DNA and RNA.
- 15 3. The method according to claim 2 wherein the natural or artificial derivatives of DNA
and RNA are selected from the group consisting of INA, ANA, MNA, PNA, LNA,
HNA, CNA, and chimeric combinations thereof.
4. The method according to claim 2 wherein the nucleic acid is genomic DNA.
5. The method according to any one of claims 1 to 4 wherein the agent is selected from
bisulfite, acetate or citrate.
- 20 6. The method according to claim 5 wherein the agent is sodium bisulfite, a reagent,
which in the presence of water, modifies cytosine into uracil.
7. The method according to any one of claims 1 to 6 wherein the INA is
phosphoramidite of (S)-1-O-(4,4'-dimethoxytriphenylmethyl)-3-O-(1-pyrenylmethyl)-
glycerol.
- 25 8. The method according to any one of claims 1 to 7 wherein the target region includes
at least one 5'-methyl cytosine in the untreated nucleic acid.
9. The method according to any one of claims 1 to 7 wherein the detector ligand is
directed to a CpG- or CpNpG-- containing region of DNA, where N designates any
one of the four possible bases A, T, C, or G.
- 30 10. The method according to claim 9 wherein the CpG-, or CpNpG- containing region of
DNA is in a regulatory region of a gene or an enhancer of any regulatory element or
region including promoter, enhancer, oncogene, retro-element, mobile or mobilisable

sequence or other regulatory element which activity is altered by environmental factors including chemicals, toxins, drugs, radiation, synthetic or natural compounds and microorganisms or other infectious agents such as viruses, bacteria, fungi and prions.

- 5 11. The method according to any one of claims 1 to 10 wherein prior to treating the sample, the nucleic acid is undergoes an enrichment or selection step.
- 10 12. The method according to claim 10 wherein the enrichment or selection step is selected from the group consisting of physical methods including sonication and shearing, enzymatic digestion, enzymatic treatment, restriction digestion, nuclease treatment, Dnase treatment, concentration, antibody capture, chemical methods including acidic or base digestion and combinations thereof.
13. The method according to claim 11 wherein the enrichment or selection step is treatment with an antibody directed to 5'-methyl cytosine so as to obtain a methylated nucleic acid sample.
- 15 14. The method according to any one of claims 1 to 13 wherein the method detects methylation of a target nucleic acid by providing to the treated sample a detector ligand in the form of an intercalating nucleic acid (INA) capable of distinguishing between methylated and unmethylated cytosine of nucleic acid, such that detection of binding of the detector ligand to the nucleic acid in the sample is indicative of the extent of methylation of the target nucleic acid.
- 20 15. The method according to any one of claims 1 to 14 wherein a capture ligand capable of recognising a first part of a target nucleic acid sequence is bound to a solid support such that the treated nucleic acid binds to the support via the first capture ligand, the bound nucleic acid is then exposed to a detector ligand capable of recognising a second part of the target nucleic acid sequence and allowing sufficient time for the detector ligand to bind to a target nucleic acid bound to a support wherein binding of the detector ligand to nucleic acid bound to the support is measured to determine the presence of the target nucleic acid in the sample, wherein at least one of the capture ligand or the detector ligand is an INA ligand.
- 25 16. The method according to claim 15 wherein the ligands are selected from the group consisting of INA probe, peptide nucleic acid (PNA) probe, LNA probe, HNA probe, ANA probe, MNA probe, oligonucleotide, modified oligonucleotide, single stranded DNA, RNA, aptamer, antibody, protein, peptide, a combination thereof, and chimeric versions thereof.
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17. The method according to claim 16 wherein the capture ligand is selected from the group consisting of INA probe, PNA probe, and oligonucleotide probe.
18. The method according to claim 15 wherein both the capture ligand and the detector ligand are an INA ligand.
- 5 19. The method according to any one of claims 15 to 18 wherein the detector ligand is an INA ligand capable of distinguishing between methylated and unmethylated cytosine of DNA and the degree or amount of binding of the detector ligand is indicative of the extent of methylation of the target nucleic acid.
- 10 20. The method according to any one of claims 15 to 19 wherein the support is selected from the group consisting of plastic materials, fluorescent beads, magnetic beads, shaped particles, plates, microtiter plates, synthetic or natural membranes, latex beads, polystyrene, column supports, glass beads or slides, nanotubes, arrays, fibres, organic, and inorganic supports.
- 15 21. The method according to claim 20 wherein the support is a magnetic bead, a fluorescent bead, a shaped particle, bead array, or a microtiter plate with one or more wells.
22. The method according to any one of claims 15 to 21 wherein a plurality of capture ligands are arrayed on the solid support.
- 20 23. The method according to any one of claims 1 to 22 wherein the INA detector ligand has a detectable label attached thereto.
24. The method according to claim 23 wherein detectable label is selected from the group consisting of chemiluminescence, fluorescence, radioactivity, enzyme, hapten, and dendrimer.
- 25 25. The method according to any one of claims 1 to 24 wherein the nucleic acid bound to the INA detector ligand is further processed or treated.
26. The method according to claim 25 wherein the nucleic acid is amplified using polymerase chain reaction using primers directed to regions of nucleic acid.
27. The method according to claim 26 wherein the primers are INA ligands.
- 30 28. A kit for analysing nucleic acid which has been treated with an agent that modifies unmethylated cytosine comprising at least one INA ligand capable of distinguishing between methylated and unmethylated cytosine of DNA.
29. The kit according to claim 28 wherein one or more INA ligands are immobilized to a solid support.

- 5 30. The kit according to claim 29 wherein the solid support is selected from the group consisting of plastic materials, fluorescent beads, magnetic beads, shaped particles, plates, microtiter plates, synthetic or natural membranes, latex beads, polystyrene, column supports, glass beads or slides, nanotubes, arrays, fibres, organic, and inorganic supports.
31. The kit according to any one of claims 28 to 30 further comprising primers for amplifying treated DNA.
32. The kit according to claim 31 wherein the primers are INA primers.